

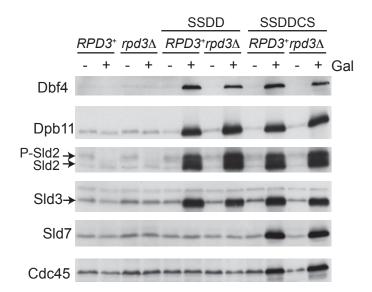
Anti-Sld2 western

Ghaemmaghami S, et al. molecules/cell
ratio of myc-protein to Orc2-13myc
endogenous protein vs 13myc
This study.

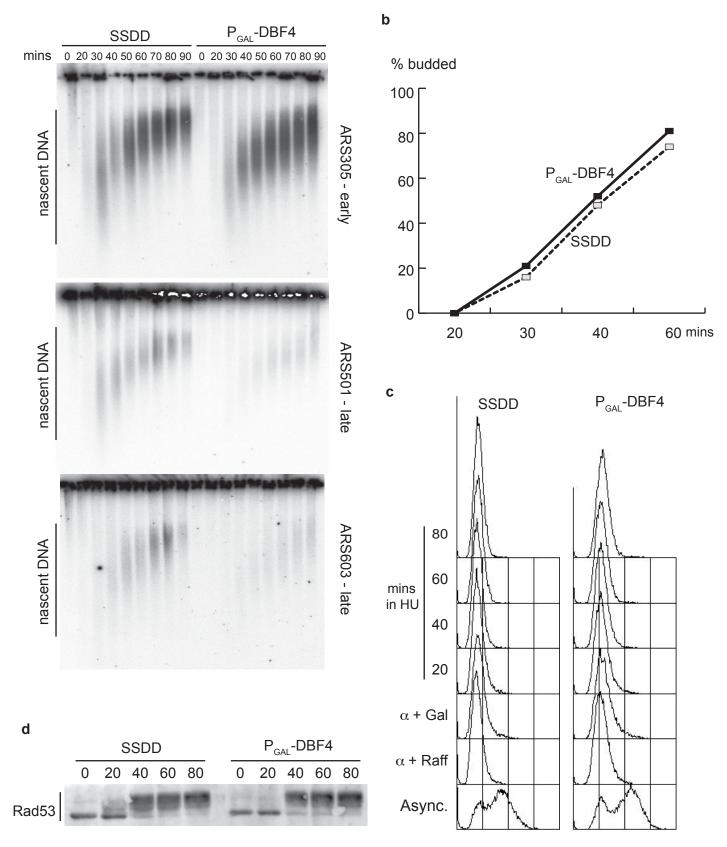
molecules/cell

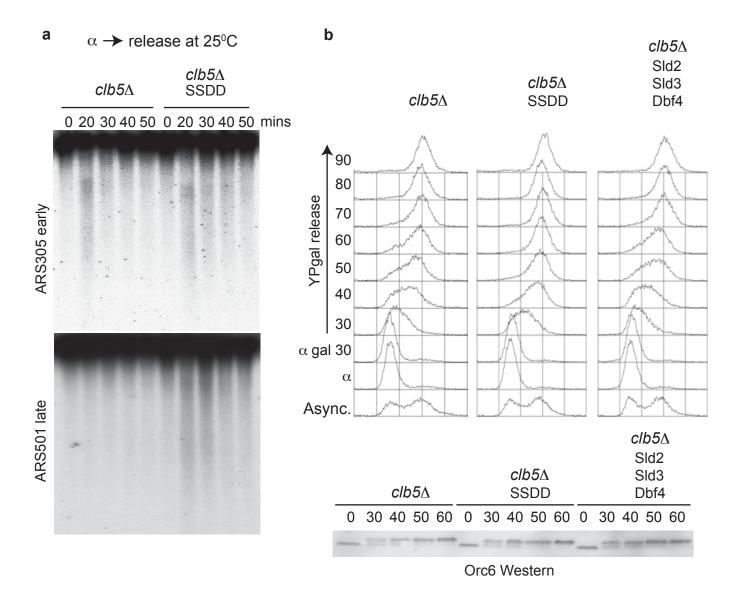
е

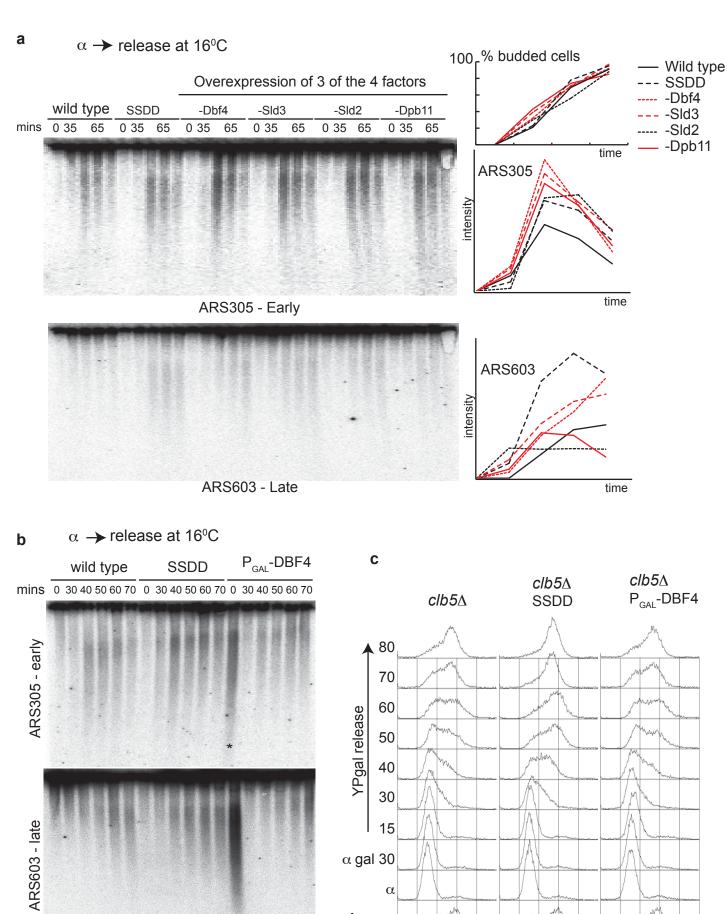
Sld3	Sld2	Dbf4	Dpb11	Sld7	Cdc45	Mcm10	Orc2
125	656	n/a	540	1690	1730	1860	1700
25-50%	25-50%	<10%	25-50%	n/d	n/d	100%	-
1:1	1:1	1:1	1:1	n/d	n/d	n/d	n/d
425	250	n/d	425	>1650	>2000	n/d	n/d



# a $\alpha \rightarrow$ release in HU

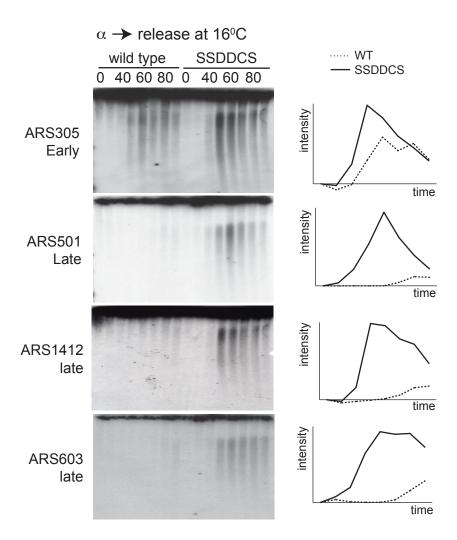


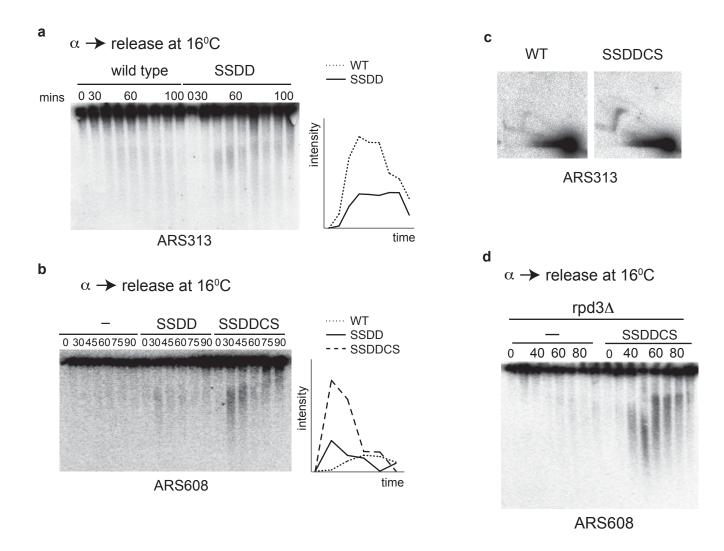


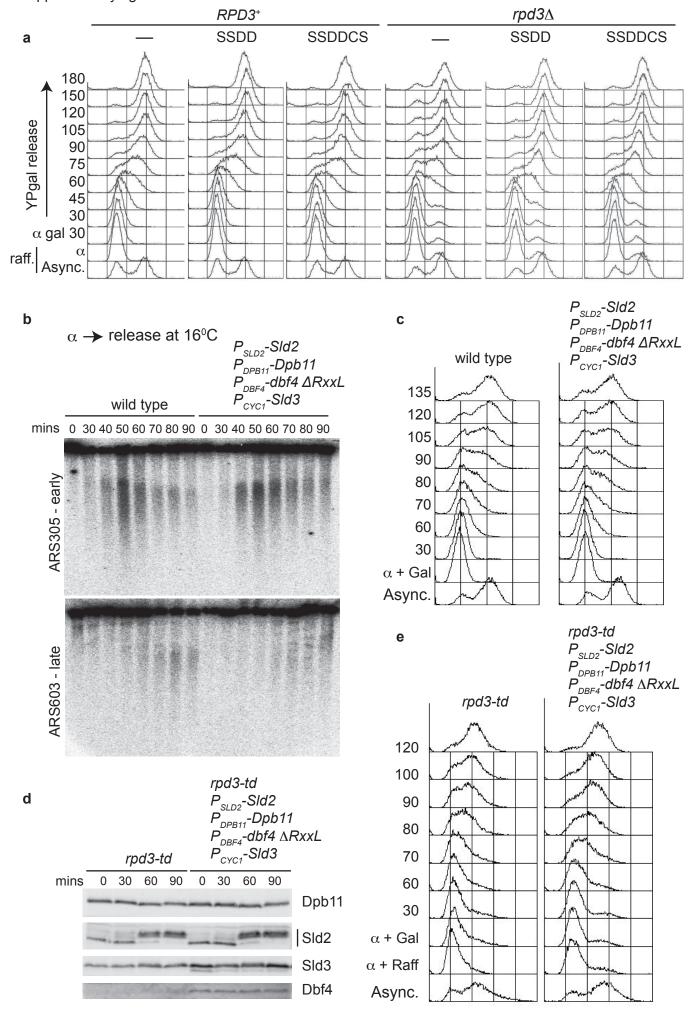


Async.

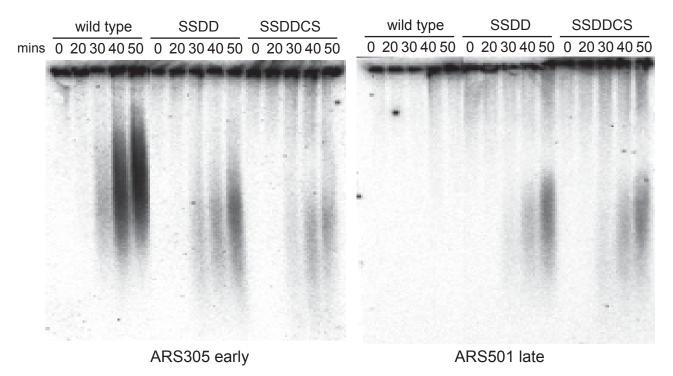
# Supplmentary Figure 7.

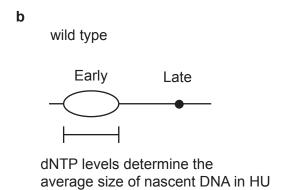


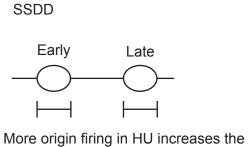




# a $\alpha \rightarrow$ release in HU

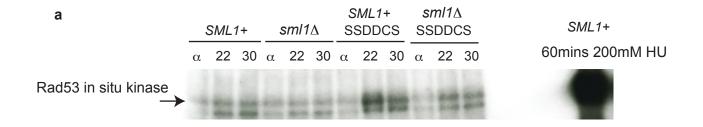


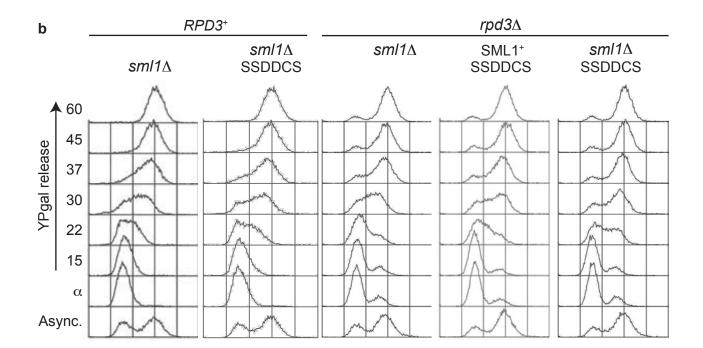


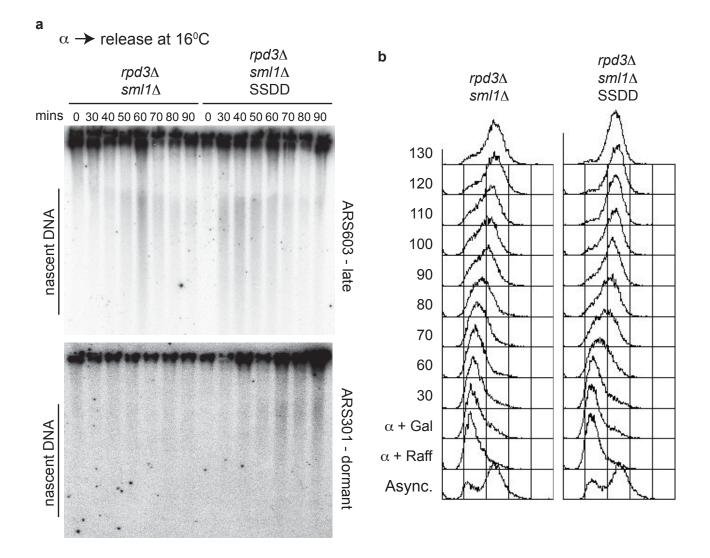


DNA is on average smaller.

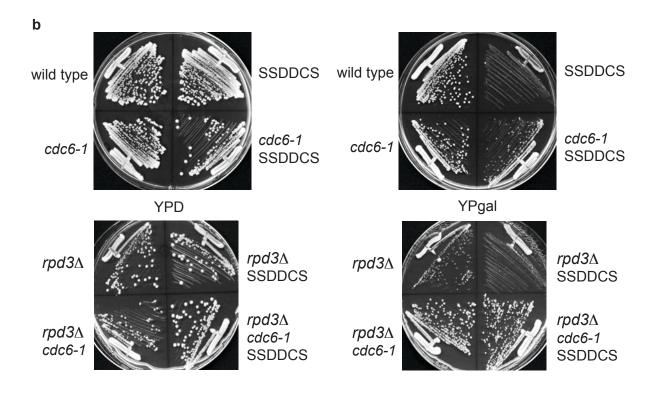
rate of dNTP depletion and nascent

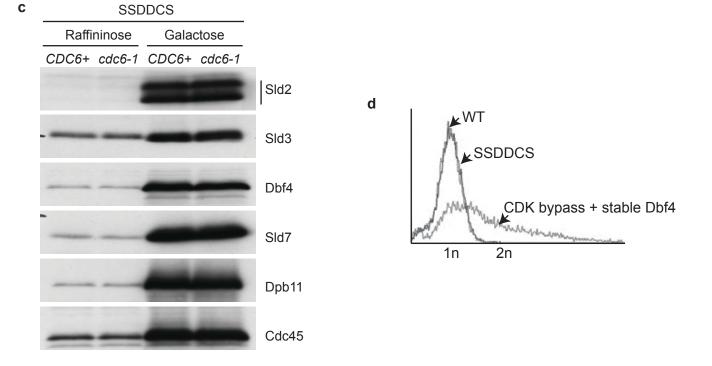












#### Supplementary Figure legends.

### Supplementary Fig. 1.

Hypothetical model of how different affinities for limiting replication initiation factors at origins can execute a temporal programme of origin firing.

Pre-RCs (yellow circles) form at all potential origins in G1 phase and yet some origins fire early, late or remain dormant during S-phase. A simple model to explain this temporal programme of origin activation is that pre-RCs have different affinities for one or more limiting replication initiation factors (X). X can only bind to the highest affinity pre-RCs in early-S phase, and is then subsequently recycled for later initiation events. This model is applicable to any rate-limiting activator that can be recycled during S-phase.

#### Supplementary Fig. 2.

Quantification of the levels of replication proteins in vivo.

a) Sld3, Sld2 and Dpb11 are less abundant than Orc2. Two-fold dilution series of 13myc tagged protein extracts from alpha factor arrested (G1) or asynchronous cells (async). The control western is anti-PSTAIRE. The ratio of myc tagged Sld2, Sld3, Dpb11 to Orc2 is included in table e). b) Comparison of Sld2-13myc levels with untagged Sld2. Anti-Sld2 western blot of a serial dilution of the 13myc tagged protein extracts versus the untagged protein extract, both from G1 arrested cells. The ratio of 13myc-tagged/untagged-protein is reported in table in e). c) Quantification of recombinant Sld2 for using as a standard for endogenous yeast Sld2. The concentration of *E.coli* expressed, recombinant his-Sld2 full length was quantified using Image J software after coomassie staining relative to BSA standard. d) Comparison of recombinant Sld2 levels with Sld2 from a known number of yeast

cells. Anti-Sld2 western blot of a serial dilution of recombinant Sld2 (rSld2) relative to endogenous yeast Sld2 in G1 phase. The number of Sld2 molecules per cell calculated with this analysis is reported in table in e). e) Summary of the results of the analyses in panels b-d, and Figure 1a, including other published work (Ghaemmaghami et al, 2003). The quantification of the number of molecules per cell for Sld3, Dpb11, Sld7 and Cdc45 was as for Sld2 described here in b-d.

### Supplementary Fig. 3.

Western blots of galactose inducible expression systems for analyzing the limiting nature of Sld3, Sld2, Dpb11 and Dbf4.

The indicated strains were grown to log-phase at 30°C in YP-raffinose medium. Galactose was then added to cell cultures at a final concentration of 2% for 30 minutes to induce protein over-expression. Protein extracts pre- (-) and post-induction (+) were analysed with the antibodies indicated on the left of each panel. The SSDD strain expresses Sld3, Sld2, Dpb11 and Dbf4 from the *GAL1-10* promoter, while the SSDDCS strain also expresses Sld7 and Cdc45 from the *GAL1-10* promoter. For both the SSDD and SSDDCS strains the Gal inducible genes are integrated at marker genes as second copies.

#### Supplementary Fig. 4.

Over-expression of Dbf4 alone causes some initiation at late origins in hydroxyurea.

**a)** Southern blot of replication intermediates from cells released from alpha factor (0) into YPgalactose + 200mM hydroxyurea at 25 °C. **b)** Budding index from experiment

in a). **c)** Flow cytometry from experiment in a). **d)** Rad53 western blot from experiment in a).

#### Supplementary Fig. 5.

Late origins fire early in *clb5* null cells when Sld3, Sld2, Dpb11 and Dbf4 are over-expressed.

a) The *clb5∆* and SSDD *clb5∆* strains were arrested in alpha-factor in YP-raffinose medium (time 0). Fresh alpha factor and 2% galactose was added to arrested cultures for a further 30 minutes. Cells were subsequently released into fresh YP-galactose medium at 25°C. Samples were collected at the indicated time points and analyzed for nascent DNA at origins *ARS305* and *ARS501* by southern blot. b) Overexpression of Sld3 and Sld2 is not sufficient to accelerate S-phase in Clb5 null cells. Top: Flow cytometry of the indicated strains at 25°C. The third strain is as the SSDD strain except that it does not express Dpb11 from the Gal promoter. Bottom: Orc6 Western blot.

### Supplementary Fig. 6.

Some advance in replication time when any three of the four limiting factors are overexpressed.

**a)** Southern blot of replication intermediates from cells released from alpha factor (0) into YPgalactose at 16°C. Time points were taken every 15 minutes from 35-80 minutes. Top right: budding index. The nascent DNA at *ARS305* and *ARS603* was quantified from the Phosphorimager files (middle- and bottom-right respectively) using Image J software. The y-axis is measured in arbitrary units. **b)** as in a) except

that the third strain expresses Dbf4 alone from the Gal promoter. Note that the DNA in the lane marked with an \* is degraded and should be disregarded. **c)** Dbf4 over-expression alone is not sufficient to accelerate S-phase in *CLB5* null cells. Flow cytometry of strains as indicated.

### Supplementary Fig. 7.

Additional over-expression of Cdc45 and Sld7 with Sld3, Dbf4, Sld2, Dpb11 increases efficiency of replication initiation at 16°C.

Southern blot of replication intermediates from cells released from alpha factor into YPgalactose at 16°C. Time points were taken every 10 minutes from 30-90 minutes after alpha factor arrest (0). Right: The nascent DNA was quantified from the Phosphorimager files using Image J software. The y-axis is measured in arbitrary units.

#### Supplementary Fig. 8.

The 'inefficient' origins *ARS608* and *ARS313* initiate replication when Sld3, Sld2, Dpb11 and Dbf4 are over-expressed.

a) Southern blot of replication intermediates at the 'inefficient' origin *ARS313* from cells released from alpha factor into YPgalactose at 16°C. Time points were taken every 10 minutes from 30 to 100 mins. Right. Graphic quantitiation of nascent DNA using Image J analysis of phosphorimage on the left. b) as in a) for *ARS608*. c) 2D N/N gel analysis of replication intermediates from S-phase of the indicated strains. Early S-phase enrichment was obtained by pooling samples taken every 5 minutes from 10 to 30 minutes after release from alpha factor into YP-galactose at 30°C. The

genomic DNA digest was Pml1 for *ARS313*. The increase in large Y intermediates in the SSDDCS strain is possibly due to increased replication initiation at neighboring origins. **d)** as in a) Time points were taken every 10 minutes from 30 to 90 mins.

### Supplementary Fig. 9.

*RPD3* inactivation combined with increased expression of Sld3, Sld2, Dpb11 and Dbf4 makes S-phase faster.

a) Full flow cytometry of experiment in Figure 4a. Strains were released from alpha factor into YPgalactose at 16°C. b) A second copy of Sld3, Sld2, Dpb11 and Dbf4 is not sufficient to make late origins fire early. Southern blot of replication intermediates from cells released from alpha factor (0) into YPgalactose at 16°C. Timepoints were taken every 10 minutes from 30 to 90 minutes after release from alpha factor (0). Sld2, Dpb11 and Dbf4 are expressed as second copies from their own promoter, while Sld3 is expressed as a second copy from the Cyc1 promoter. The *dbf4* Δ*RxxL* allele corresponds to the APC/C destruction box mutations R10A, L13A and R62A, L65A. c) Flow cytometry from the experiment in b). d) Western blot analysis of Rpd3 temperature degron strains containing a second copy of Sld3, Sld2, Dpb11 and Dbf4. e) *RPD3* inactivation combined with increased dosage of Sld3, Sld2, Dpb11 and Dbf4 makes S-phase faster. Flow cytometry of experiment in Figure 4b.

### Supplementary Fig. 10.

The early firing of late origins reduces the average size of nascent DNA at origins in hydroxyurea. **a)** Southern blot of replication intermediates from cells released from alpha factor into YPgalactose + 200mM HU at 25°C. Time points were taken every 10 minutes from 20-50 minutes after alpha factor arrest (0). The SSDDCS and SSDD

strains both cause the firing of late origins in HU. **b)** Schematic diagram to explain how dNTP levels affects the average size of nascent DNA in hydroxyurea and how this is further affected by increased origin firing. In hydroxyurea, forks stall at a distance from origins that is proportional to the amount of nucleotides available. When extra origins fire early this depletes nucleotides at a faster rate and on average forks stall earlier.

### Supplementary Fig. 11.

The early firing of origins causes checkpoint activation that is suppressed by increased nucleotide levels.

**a)** In situ Rad53 kinase assay of the indicated strains released from alpha factor into YPgalactose at 25°C. The lane on the right is from a wild type strain arrested for 60 mins in 200mM HU. Note that this is the same gel and the same exposure. From this it is clear that the amount of stochastic fork stalling in the SSDDCS strain is much lower than in HU. **b)** Increased nucleotide concentration in *SML1* null cells further increases the speed of S-phase in cells that both lack Rpd3 and over-express the limiting factors. Full flow cytometry from experiment in Figure 5c.

#### Supplementary Fig. 12.

The early firing of late origins and the firing of dormant origins in the  $rpd3\Delta$  SSDD strain is not due to transient checkpoint activation.

a) Southern blot of replication intermediates from cells released from alpha factor (0) into YPgalactose at 16°C. b) Flow cytometry of experiment in a).

#### Supplementary Fig. 13.

Deregulation of the temporal programme of origin firing causes loss of viability.

a) Serial dilutions of cell cultures of the indicated strains were spotted on YPD and YP-galactose plates and incubated at 30°C for 2 days. b) Single colonies of the indicated strains were streaked on YPD and YP-galactose plates and incubated at 30°C for 2 days. c) The SSDDCS and SSDDCS *cdc6-1* strains were grown to log-phase at 30°C in YP-raffinose medium. Galactose was then added to cell cultures at a final concentration of 2% to induce protein over-expression for 30 minutes. Protein extracts pre- (-) and post-induction (+) were analysed with the antibodies indicated on the right of each panel. d) Overexpression of Sld3, Sld2, Dbf4, Dpb11, Cdc45 and Sld7 does not cause detectable levels of re-replication in G1 phase by flow cytometry. The CDK bypass strain has the Sld3-Dpb11 fusion expressed from the *SLD3* promoter and *sld2 T84D* expressed from the *GAL1-10* promoter (Zegerman & Diffley, 2007). The *dbf4 ΔRxxL* allele corresponds to the APC/C destruction box mutations R10A, L13A and R62A, L65A. For this experiment the strains were held in alpha factor for 4 hours in galactose.

Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS (2003) Global analysis of protein expression in yeast. *Nature* **425**(6959): 737-741

Zegerman P, Diffley JF (2007) Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. *Nature* **445**(7125): 281-285

**Supplementary Materials and Methods.** 

**Over-expression experiments.** All the time-course experiments (with the exception of the dense isotope substitution experiments) that involve the over-expression of any or all the SSDDCS factors (*DPB11*, *SLD2*, *SLD3*, *DBF4*, *CDC45* and *SLD7*) from the galactose promoter were performed with the following scheme. Cell-cultures of the appropriate strains exponentially growing in YP-raffinose at 30°C were synchronized in G1 with 5μg/ml alpha-factor. When more than 95% cells were arrested in G1-phase 2% galactose was added to the cell cultures. After 30 minutes of induction, cells were washed and released in YP-galactose medium without alpha-factor to allow entry into S-phase with high levels of the over-expressed proteins. For some experiments the cells were released in the presence of hydroxyurea, and the release temperature varies according to the experiment (see figure legends for these details).

Analysis of Nascent DNA (Replication Intermediates) at origins of DNA replication. This technique was performed as previously described with minor modifications<sup>1</sup>. The probes were generate by radioactive labeling PCR fragments corresponding to specific ARSs using the Prime-a-Gene® Labeling System (Promega). The PCR fragments for each ARS were obtained using the following primers:

**ARS301** 

PZ954 5'-GAATCAAATAGGTGTATCGC-3'

PZ955 5'-GTCTCAAGAAGAGTTAACAA-3'

ARS302/303/320

PZ936 5'-GGATTGTATTTCTATTGAC-3'

PZ937 5'-GGGTGGAAACGAATGGATG-3'
ARS305
PZ875 5'-CCTAGTGGATCCTCTCCTTC-3'
PZ876 5'-CGCTAACGAAGACAATTTAG-3'
ARS313
PZ942 5'-CTCACCTTTCAAGGCCATGC-3'
PZ943 5'-GTAAGGCAGTTTCATCTTCAG-3'
ARS501
PZ979 5'-GGATCCCGAGTCATGTTTGG-3'
PZ980 5'-GAGCATAATTATGACTGTAG-3'
ARS603
PZ746 5'-GGTATTGCTGTTTTAAGTGAG-3'
PZ747 5'-CATAGATATCGGGTTACTAAAG-3'
ARS608
PZ944 5'-CCATCTGTTATGAAATTACC-3'
PZ945 5'-GCAGACCTTTTACCAAATTC-3'
ARS1412
PZ981 5'- CACCGAGGAACTATTCGGTG-3'
PZ982 5'- ACTGAAGCACATAGAGCGTC-3'.

ARS310

PZ1009 5' - TATAACTTCATGTCTTTTCT - 3'

PZ1010 5' - TGCTCCTTTTGAAACAAATG - 3'

ARS316

PZ1011 5' - CTTAAAGGAATGAAAGAATC - 3'

PZ1012 5' - CATTAGTAGTAGCATACCTT - 3'

After probing the membrane was exposed to a Phosphorimager screen, scanned and the signal corresponding to the nascent DNA was quantified using the Image-J software.

2-dimensional Neutral/Neutral gel electrophoresis.

This technique was performed as previously described (1)

Calculation of the number of molecules per cell of replication proteins. As a first step each protein was expressed from *E. coli* and its concentration was estimated by comparing it with BSA standards of known concentrations. Serial dilutions of the recombinant protein, corresponding to a known number of molecules, were then analyzed by western blot side by side with serial dilutions of yeast protein extracts obtained from a known amount of cells. The signals given by the endogenous protein were matched with signals of the same intensity given by recombinant protein to obtain an estimate of the number of molecules per cell.

Zegerman, P. & Diffley, J. F. Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation. *Nature* **467**, 474-478 (2010).

Table of S. cerevisiae strains used in this study

Strain	Relevant genotype	source
W303a	MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100	-
PZ590	W303a ORC6-13MYC::KanMX	this work
PZ521	W303a ORC2-13MYC::KanMX sml1∆::URA3	this work
PZ585	W303a MCM10-13MYC::KanMX	this work
PZ574	W303a MCM4-13MYC::KanMX sml1∆::URA3	this work
PZ575	W303a MCM6-13MYC::KanMX sml1∆::URA3	this work
PZ168	W303a SLD2-13MYC::KanMX sml1Δ::URA3	this work
PZ520	W303a SLD3-13MYC::KanMX sml1∆::URA3	this work
PZ452	W303a DPB11-13MYC::KanMX sml1∆::URA3	this work
PZ559	W303a POL1-13MYC::KanMX sml1∆::URA3	this work
PZ560	W303a PRI2-13MYC::KanMX sml1∆::URA3	this work
PZ561	W303a POL3-13MYC::KanMX sml1∆::URA3	this work
PZ562	W303a POL31-13MYC::KanMX sml1∆::URA3	this work
PZ565	W303a DPB2-13MYC::KanMX sml1∆::URA3	this work
PZ519	W303a DBF4-13MYC::KanMX sml1∆::URA3	this work
1366	W303a CDC7-13MYC::KanMX	this work
PZ529	W303a his3::pGAL <sub>1-10</sub> -SLD3-13MYC::HIS3 trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 ura3::pGAL <sub>1-10</sub> -DPB11::URA3 leu2::pGAL <sub>1-10</sub> -DBF4-2HA::LEU2	this work
PZ130	W303a his3::pGAL <sub>1-10</sub> -SLD3-13MYC::HIS3 trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 ura3::pGAL <sub>1-10</sub> -DPB11::URA3	this work
PZ598	MATa ADE2 URA3 his3-11,15 trp1-1 leu2-3,112 can1-100	A. Donaldson
AMY9	MATa ADE2 URA3 his3-11,15 trp1-1 leu2-3,112 can1-100 trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3	A. Donaldson
PZ629	W303a clb5∆::URA3	this work
PZ665	W303a clb5∆::URA3 trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3	this work
	I .	

PZ496	W303a trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3	this work
PZ498	W303a trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ141	W303a his3::pGAL <sub>1-10</sub> -SLD3-13MYC::HIS3 trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 ura3::pGAL <sub>1-10</sub> -DPB11::URA3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2 DBF4::pGAL <sub>1-10</sub> -DBF4::KanMX	this work
PZ477	W303a rpd3∆::HphMX	this work
PZ580	W303a rpd3∆::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL1-10-dbf4A::HIS3	this work
PZ488	W303a rpd3∆::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ356	W303a sml1Δ::URA3	this work
PZ523	W303a sml1Δ::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ523	W303a sml1∆::KanMX rpd3∆::HphMX	this work
PZ494	W303a sml1Δ::URA3 rpd3Δ::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ674	W303a $sml1\Delta$ ::URA3 $rpd3\Delta$ ::HphMX $trp1$ ::SLD2- $pGAL_{1-10}$ -DPB11::TRP1 $his3$ ::sld3A- $pGAL_{1-10}$ -dbf4A::HIS3	this work
PZ131	W303a his3::pGAL <sub>1-10</sub> -SLD3-13MYC::HIS3 trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 ura3::pGAL <sub>1-10</sub> -DPB11::URA3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ511	W303a his3::pGAL <sub>1-10</sub> -DBF4-2HA::HIS3 trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 ura3::pGAL <sub>1-10</sub> -DPB11::URA3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ505	W303a his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2 ura3::pGAL <sub>1-10</sub> -DPB11::URA	this work
PZ504	W303a his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2 trp1::pGAL <sub>1-10</sub> -SLD2::TRP1	this work

PZ507	W303a trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::pGAL <sub>1-10</sub> -SLD7::LEU2	this work
PZ539	W303a trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 ura3::pGAL <sub>1-10</sub> -CDC45::URA3	this work
PZ621	W303a cdc6-1	K. Nasmyth (Piatti et al. 1995)
PZ642	W303a cdc6-1 trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3 leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ648	W303a rpd3∆::HphMX cdc6-1	this work
PZ649	W303a rpd3\(\Delta\)::HphMX cdc6-1 trp1::SLD2-pGAL1-10-DPB11::TRP1 his3::sld3A-pGAL1-10-dbf4A::HIS3 leu2::SLD7-pGAL1-10-CDC45::LEU2	this work
PZ661	W303a sld3 <sup>800/609/622A</sup> -DPB11 <sup>253-764</sup> ::KanMX his3::pGAL <sub>1-10</sub> -sld2 <sup>D84</sup> ::HIS3 leu2::p <sub>DBF4</sub> -dbf4 <sup>RxxL</sup> ::LEU2	Zegerman et al, 2007 Nature
PZ699	W303a trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 ura3::SLD3-pGAL <sub>1-10</sub> -DBF4::URA3	this work
PZ700	W303a rpd3∆::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 ura3::SLD3-pGAL <sub>1-10</sub> -DBF4::URA3	this work
PZ701	W303a trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 ura3::SLD3-pGAL <sub>1-10</sub> -DBF4::URA3 leu2::SLD7-pGAL1-10-CDC45::LEU2	this work
PZ702	W303a rpd3\(\Delta\)::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 ura3::SLD3-pGAL <sub>1-10</sub> -DBF4::URA3 leu2::SLD7-pGAL1-10-CDC45::LEU2	this work
PZ4	W303a dbf4∆::TRP1 his3::p <sub>DBF4</sub> -dbf4 <sup>19A</sup> ::HIS3 sld3 <sup>37A</sup> -10HIS-13MYC::KanMX	Zegerman et al, 2010 Nature
PZ530	W303a trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 ura3::pGAL <sub>1-10</sub> -DPB11::URA3 leu2::pGAL <sub>1-10</sub> -DBF4-2HA::LEU2	this work
PZ531	W303a his3::pGAL <sub>1-10</sub> -SLD3-13MYC::HIS3 ura3::pGAL <sub>1-10</sub> -DPB11::URA3 leu2::pGAL <sub>1-10</sub> -DBF4-2HA::LEU2	this work

PZ538	W303a his3::pGAL <sub>1-10</sub> -SLD3-13MYC::HIS3 trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 ura3::pGAL <sub>1-10</sub> -DBF4::URA3	this work
PZ578	W303a leu2::pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ576	W303a leu2::SLD7-pGAL <sub>1-10</sub> -CDC45::LEU2	this work
PZ581	W303a rpd3∆::HphMX trp1::pGAL <sub>1-10</sub> -SLD2::TRP1 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3	this work
PZ582	W303a rpd3∆::HphMX ura3::pGAL <sub>1-10</sub> -DPB11::URA3 his3::sld3A-pGAL <sub>1-10</sub> -dbf4A::HIS3	this work
PZ584	W303a rpd3∆::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 leu2::pGAL <sub>1-10</sub> -DBF4-2HA::LEU2	this work
PZ583	W303a rpd3∆::HphMX trp1::SLD2-pGAL <sub>1-10</sub> -DPB11::TRP1 leu2::pGAL <sub>1-10</sub> -SLD3-13MYC::LEU2	this work
PZ843	W303a RPD3::PCUP1::rpd3-td::URA3 SLD3 5'UTR::KanMx-tTA-tetO2::SLD3 trp1:: P <sub>DPB11</sub> -DPB11::TRP1 leu2::P <sub>DBF4</sub> -dbf4-RxxL::LEU2 his3:: P <sub>SLD2</sub> -SLD2::HIS3	this work
PZ840	W303a RPD3::PCUP1::rpd3-td::URA3	this work
PZ751	W303a SLD3 5'UTR::KanMx-tTA-tetO2::SLD3 trp1:: P <sub>DPB11</sub> -DPB11::TRP1 leu2::P <sub>DBF4</sub> -dbf4-RxxL::LEU2 his3:: P <sub>SLD2</sub> -SLD2::HIS3	this work
PZ766	MATa clb5Δ::KanMX ura3::SLD3-P <sub>GAL1-10</sub> -DBF4::URA3 his3::P <sub>GAL1-10</sub> -SLD2::HIS3	this work
PZ788	MATa clb5Δ::KanMX ura3::P <sub>GAL1-10</sub> -DBF4-3HA::URA3	this work